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Comparison of physicochemical characteristics of cassava starch modified by ultrasonication and oxidation with ozone

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Abstract

Cassava starch was modified chemically using dissolved ozone (oxidation with ozone) and physically using ultrasonication. Oxidation with ozone and ultrasonication are advanced and environmentally friendly oxidation methods. Determination of the starch oxidation method should be considered to meet consumer needs and safety. Comparison between two starch modification methods (oxidation with ozone and ultrasonication) is still limited. Therefore, this work aimed to compare two environmental-friendly methods by evaluating the carbonyl and carboxyl contents, amylose content, brightness, pasting and functional properties, spectrum profiles of Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), and SEM of modified starch with ozone and ultrasonication. Oxidation with ozone was performed by combining the slurry concentration (w/w) and dissolved ozone concentration (ppm) taken in three combinations: 1:6 (0.8 ppm), 1:8 (2 ppm), and 1:10 (1.4 ppm). Ultrasonication combined amplitude (%) and duration process (min) was produced in three combinations: 60% (40 mins), 80% (10 mins), and 80% (30 mins). The results showed that the degree of oxidation and the physicochemical characteristics of cassava starch were significantly more affected by ozone than ultrasonication. However, the ultrasonication method effectively depolymerized starch, resulting in amylose content and paste viscosity almost equivalent to those achieved by oxidation with ozone. Considering the safety and environmental-friendly methods for starch modification, both oxidation with ozone and ultrasonication can be the preferred method for obtaining starch with characteristics as needed.

1. Introduction

Starch is the main component of several grains, representing 70-80% of their composition, mainly found in roots and tubers which consist of two main components: amylose and amylopectin. Starch is considered a biopolymer due to its natural origin, which consists of two main components: amylose and amylopectin. Amylose is an essentially linear polymer composed of glucose units joined by α-(1-4) bonds and a few branching points conforming amorphous regions of the starch granules. On the other hand, amylopectin is a branched polymer, in which the glucose units are also joined by α-(1-4) bonds in the linear sections, and by α-(1-6) bonds in the branching points. Amylopectin is responsible for the starch crystalline lamella, although their branching points are part of the amorphous one. The large molecular size and branching of amylopectin molecules reduce solubility and weaken intramolecular bonds of the starch (Castanha *et al*., 2017). Native starch has a characteristic which is limited to being applied in food products (low solubility, less attractive color, unstable paste viscosity while undergoing a heating process for a specified time or in cold conditions). Modification of starch aims to increase its usefulness by changing the characteristics of native starches such as reducing viscosity, increasing the solubility, and enhancing physical appearance (attractive colors). Modified starch provides beneficial functional properties that add value to the starch itself (Kaur *et al*., 2012).

Chemical modification of starch remains an option due to its ease of control and comprehensible chemical mechanism compared to other starch modification methods (Fouladi and Mohammadi Nafchi, 2014). A chemical modification that is safe is oxidation with ozone. The pro-oxidant effects of ozone have been

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widely documented and oxidation with different ozone generation times (OGT) showed structural and properties changes on corn, sago, and cassava starches with the enhancement of carboxyl groups number and degradation of starch granules (Chan *et al*., 2012; Klein *et al*., 2014). Apart from the chemical modification methods, there are also physical modification methods that are environmentally friendly such as ultrasonication (Çatal and İbanoğlu, 2014). The effects of ultrasonication result in the physical degradation of starch granules with visible cracks and pores on the surface while preserving the shape of starch granules (Sujka, 2017). Previous studies showed that ultrasonication also had the potential for oxidation due to the production of OH radicals through the breakage of water molecules (Milne *et al*., 2013; Miljevic *et al*., 2014). Based on the opportunity to meet the growing industrial demand while considering environmental and food security concerns, oxidation with ozone and ultrasonication to improve the characteristics of the starch are necessary. However, the use of a combination of two methods is considered to be inefficient. Based on the study conducted by Castanha *et al*. (2019), oxidation with ozone alone can significantly change the characteristics of the starch. Besides, the comparison between the oxidation effects of those two modification methods with various conditions of oxidation with ozone and ultrasonication is still lacking. This study aims to modify cassava starch using dissolved ozone and ultrasound technology. Some physicochemical characteristics of modified starches are evaluated, explained, and compared to understand the level of different methods of starch modification.

2. Materials and methods

2.1 Materials

Cassava starch is made from cassava (var. adira-4) with a harvest age of 10 to 11 months which was obtained from Yogyakarta, Indonesia. Maximum starch extraction yield (30-35%) was achieved by adjusting the ratio of cassava pulp to water 1:4 (w/w) through stripping, grating, dissolving, extracting, filtering, settling, and drying. The drying process was conducted in a cabinet drier at 50°C for 24 hrs to reach a moisture level of 9-10%. The dried starch was ground in a mortar, sieved through a 60-mesh sieve, and then packed in plastic packaging with silica gel. The packaging was wrapped in aluminum foil and placed into a container for storage at room temperature, ready for further use.

2.2 Oxidation with ozone process

Ozone produced by the generator through corona discharge methods initiates oxygen-free radical formation that leads to low ozone concentration (0.1-2 ppm) (Kim *et al*., 1999; An and King, 2009). The N,N-diethyl-p-phenylenediamine (DPD) indicator test kit was used to determine dissolved ozone concentration. The DPD indicator reacted with ozone to produce pink color with a certain color intensity (Buchan *et al*., 2005). Various conditions (starch slurry ratio and ozone concentration) 1:6 (0.8 ppm), 1:8 (2 ppm), and 1:10 (1.4 ppm) were selected for oxidation with ozone. All processes were carried out for 5 mins, precipitated, and dried at a temperature of 50°C for 24 hrs. Dried modified starch was then ground, sieved through 60-mesh sieve, and stored for further analysis.

2.3 Ultrasonication process

Ultrasound process used ultrasonication probe-type UP200St Hielscher Germany. Suspension of cassava starch (5% w/v) was kept in a glass cell and homogenized with continuous stirring for 5 mins prior ultrasonication at a constant temperature of 20±1°C. The ultrasonication process was carried out using conditions (amplitude and time duration) of 60% (40 mins), 80% (10 mins), and 80% (30 mins). After ultrasonication process, the starch suspension was precipitated and dried at a temperature of 50°C for 24 hrs. Dried modified starch was then ground, sieved through a 60-mesh sieve, and stored for further analysis.

2.4 Carbonyl, carboxyl, and amylose contents

2.4.1 Carbonyl contents

The carbonyl content was determined according to the procedure described by Wang (2003). Starch (4 g) was added to 100 mL of distilled water and stirred. The suspension was then placed in a boiling water bath for 20 min until gelatinization occurred. The suspension was cooled to 40°C and pH was subsequently adjusted to pH 3.2 using 0.1 N HCl. Hydroxylamine reagent (15 mL) was added and the sample was then placed in a water bath (40°C) with gentle stirring for 4 hrs. The excessive hydroxylamine was quantified by rapidly titrating the reaction mixture with standardized 0.1 N HCl to obtain a pH of 3.2. A blank was prepared in the same manner. Carbonyl content was calculated as follows:

$$\text{Carbonyl content (\%)} = \frac{(\text{Blank} - \text{Sample})\ \text{mL} \times \text{Normality of HCl} \times 0.028 \times 100}{\text{Sample weight (dry basis in g)}}$$

2.4.2 Carboxyl contents

The carboxyl content was determined using the method described by Chattopadhyay *et al*. (1997). Starch (2 g) was mixed with 25 mL of 0.1 M HCl and the suspension was then incubated for 30 mins under constant stirring. The mixture was then vacuum-filtered through filter paper, and distilled water was used to wash the chlorine residue. The starch cake was suspended in distilled water (300 mL) and gelatinized in a water bath

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for 15 mins with constant stirring. The sample was then immediately titrated with 0.1 M NaOH until a pH of 8.3 was obtained. A native sample was used as a blank and subject to similar treatment. The result could be calculated as follows:

$$\text{Carboxyl contents (\%)} = \frac{(\text{Blank} - \text{Sample})\ \text{mL} \times \text{Normality of NaOH} \times 0.045 \times 100}{\text{Sample weight (dry basis in g)}}$$

2.4.3 Amylose contents

Amylose content was determined using the procedure described by Association of Official Analytical Chemists (AOAC) International (1995). The amylose content was calculated from a standard curve equation prepared using pure amylose with the concentration of 0.4, 0.8, 1.2, 1.6, and 2.0% and was expressed as a percentage.

2.5 Functional properties of native and modified starch

2.5.1 Swelling power and solubility

Sample swelling power was determined based on the method described by Adebowale *et al*. (2002). A starch sample (1.0 g) was weighed and placed into a clear dried test tube, which had been weighed. The sample and tube weight were calculated as W1. Distilled water (50 mL) was added to the sample until a slurry formed. The mixture was homogenized and heated at 95°C for 30 mins in a water bath. The mixture was cooled until the temperature of 30±2°C was obtained and centrifuged (15 mins) until the gel and aliquots were separated. Aliquots (5 mL) were dried at 110°C until a constant weight was reached. The residue obtained after drying the supernatant could be expressed as the amount of starch solubilized in water. Solubility was calculated as g per 100 g of starch on a dry weight basis. The gel and test tube from the above experiment (after centrifugation) with its retained water were quantitatively weighed and taken together as W2. The swelling power was calculated as follows:

$$\text{Swelling power (\%)} = \frac{\text{W2} - \text{W1}}{\text{Sample weight (dry basis in g)}}$$

2.5.2 Water holding capacity

Water holding capacity (WHC) was determined using the method described by Uzomah and Ibe (2011). The starch sample (1 g) was placed in a dried test tube. The sample and tube were weighted as W1. Water (10 mL) was then added to the sample homogenized until the mixture was formed. The mixture was left for 30 mins prior to centrifugation (3500×*g*, 15 mins) and the residue was obtained. The tube and the residue were weighed as W2. WHC and OHC were calculated with the equation as follows:

$$\text{WHC (\%)} = \frac{\text{W2} - \text{W1}}{\text{Sample weight (dry basis in g)}}$$

2.5.3 Brightness

Brightness is measured using the Chromameter CR-tool 400/410 Conica Minolta. Sample was placed in the glass jar and was then recorded with the equipment as L*, a*, and b*. L* was expressed as brightness.

2.5.4 Pasting properties

Pasting properties were measured using Rapid Visco Analyser (RVA; 4500 Perten Instrument-Australia), and the result was expressed in cP with pasting temperature, peak viscosity, breakdown, final viscosity, and setback as parameters according to the method of Gozé *et al*. (2016). Water (25 mL) was added to the sample which had been weighed directly in the aluminium RVA canister. The temperature of the sample was held at 50°C for 1 min, increased to 95°C in 3.7 mins, and held at 95° C for 2.5 mins. The temperature of the sample was then decreased to 50°C in 3.8 mins and then held at 50°C for 2 mins. The rotating speed was held at 960 rpm for 10 s, 160 rpm for the remainder of the process.

2.6 FTIR spectroscopy analysis

The infrared spectra of the native and modified starches in the region of 4000-650 cm^{-1} were obtained with Thermo Scientific Nicolet iS-10. Dried starch samples (without KBr) were directly placed on the diamond sensor of the instrument. Three readings were collected at a resolution of 4 cm^{-1} (Monroy *et al*., 2018). All spectra were analyzed using Thermo Fisher Scientific Omnic analysis software and Origin 2016.

2.7 X-Ray diffractions

X-ray identification was carried out with an X-ray diffractometer (Rigaku Miniflex 600 RXD, Tokyo, Japan). The operation angle was 3 to 45° for 2θ using the working conditions: (1) scan rate of 2°/min and (2) 40 kV and 15 mA. Several methods were used to calculate the relative crystallinity (RC) of the starch granules using the Origin software 2016 for graphing and analysis (Srichuwong *et al*., 2005; Rocha *et al*., 2008; Katsumi *et al*., 2015).

2.8 Scanning electron microscopy

The appearance of a granular surface was determined using a scanning electron microscope (SEM JEOL-JSM-6510, JEOL Ltd. Tokyo Japan). The starch sample was placed in a single-layer position on carbon type. A sample was then coated using platinum and examined under the SEM set at a wide distance (WD) of 11 mm, spot size (SS) of 40, and voltage of 15 kV.

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2.9 Statistical analysis

A completely randomized design was used as the research design. The data were grouped into two different groups (oxidation with ozone and ultrasonication) and analyzed using one-way analysis of variance (ANOVA). Duncan Multiple Range Test was used for further analysis if there was any significant difference at a 95% confidence level. Statistical Product and Service Solution (SPSS) version 17 was used for data analysis.

3. Results and discussion

3.1 Carbonyl and carboxyl content

Starch is rich in hydroxyl groups which can be substituted by other groups through the oxidation process. Starch oxidation begins with the formation of the carbonyl groups and further changes into carboxyl groups mainly take place at C-2 and C-3. The substitution of these groups is expected during the oxidative process. The proportion of carboxyl and carbonyl groups on oxidized starches indicates the extent of oxidation (Castanha *et al*., 2017).

Both oxidation with ozone and ultrasonication can produce abundant hydroxyl radical (•OH) through ozone reaction with water or the breakage of water molecules due to ultrasonication. This study showed that dissolved ozone was more effective in generating carbonyl groups compared to the ultrasonication process, as shown in Figure 1.

The results showed that ozone concentration significantly affects carbonyl and carboxyl content while the ultrasonication process did not produce carboxyl groups significantly. This could explain that the mechanism of the oxidation process with ozone involves the release of H ions, electron transfer, and reaction with ozone radicals (Vanier *et al*., 2017). Ozone can oxidize various compounds in water by direct reactions and indirect reactions. The direct reaction is carried out by ozone itself which is dissolved in water. Whereas the indirect reaction occurs through the production of hydroxyl radical (OH) due to the decomposition process due to the oxidation reaction namely initiation, propagation, and termination (Rodríguez *et al*., 2008), thus oxidation takes place more effectively with the production of carboxyl groups than ultrasonication.

3.2 Amylose content

Oxidation with ozone and ultrasonication process significantly affected the amylose content of modified cassava starch. As the slurry concentration increases, the reaction between ozone and starch particles becomes more effective due to the possible movement of starch in the solution which is greater so that starch depolymerization can be more intensive (El-sheikh *et al*., 2010). This could be related to the depolymerization of the starch molecular chain. The oxidation of the starch granules was demonstrated by the evidence of the hydrolysis of the glycosidic linkages (the covalent bonds between the α-D-glucopyranosyl groups).

This hydrolysis generates depolymerization of the amylose and amylopectin, leading to the formation of smaller size chains (Castanha *et al*., 2017) The amylose content of native and modified starches can be seen in Figure 2.

In this study, modified starch with ultrasonication produced higher amylose content than modified starch with ozone. Ultrasonication has a significant effect on amylose levels. This can be related to the depolymerization of starch molecular chains. Oxidation of starch granules can also occur resulting in the hydrolysis of the glycosidic bond (covalent bond between α-D-glucopyranosyl units). Starch hydrolysis causes depolymerization of amylose and amylopectin molecules, leading to the formation of chains with smaller molecular weights (Castanha *et al*., 2017).

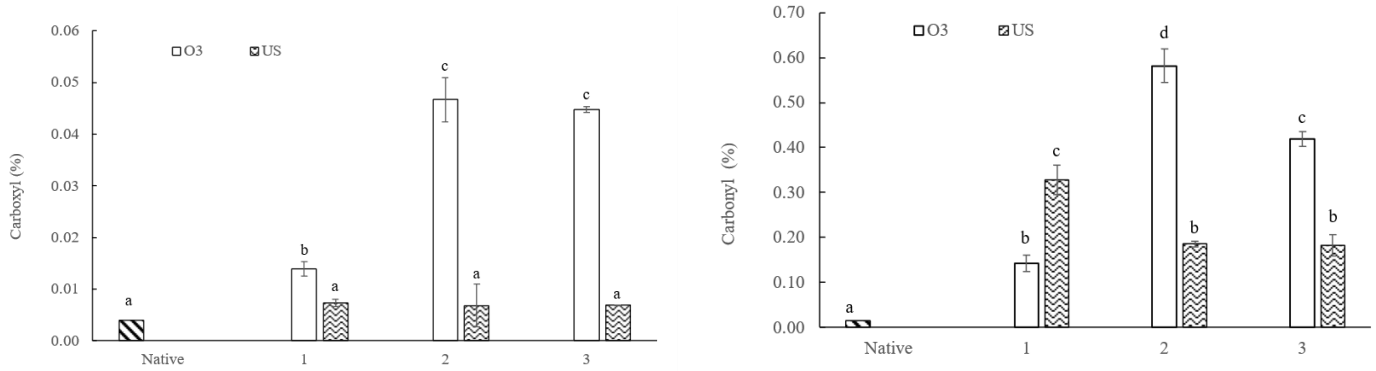

Figure 1. Carbonyl and carboxyl content of native and modified starch. Bars with different notations of the same parameter are statistically significantly different at 5% probability level. X-axis 1: starch slurry ratio 1:6 - 0.8 ppm ozone (O_3) and ultrasonication (US) 60% amplitude for 40 mins, x-axis 2: starch slurry ratio 1:8 - 2 ppm ozone and ultrasonication 80% amplitude for 10 mins, x-axis 3: starch slurry ratio 1:10 - 1.4 ppm ozone and ultrasonication 80% amplitude for 30 mins.

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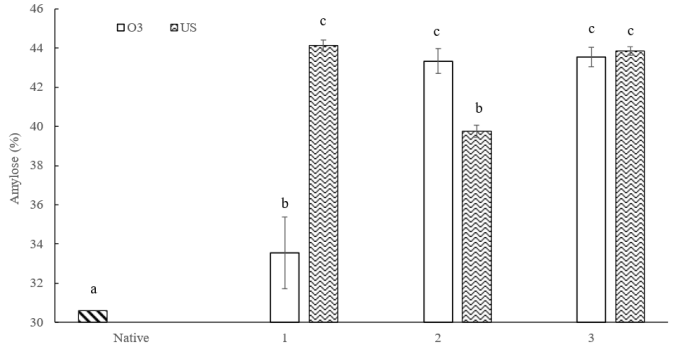

Figure 2. Amylose content of native and modified starch. Bars with different notations of the same parameter are statistically significantly different at 5% probability level. X-axis 1: starch slurry ratio 1:6 - 0.8 ppm ozone (O_3) and ultrasonication (US) 60% amplitude for 40 mins, x-axis 2: starch slurry ratio 1:8 - 2 ppm ozone and ultrasonication 80% amplitude for 10 mins, x-axis 3: starch slurry ratio 1:10 - 1.4 ppm ozone and ultrasonication 80% amplitude for 30 mins.

3.3 Swelling power and solubility

Physical, chemical, or enzymatic modification techniques could alter the hydration and swelling property by changing the magnitude of the interaction between glucan chains, the availability of free hydroxyl groups within the crystalline and amorphous regions, polymorphic pattern, nature of the crystalline region, or hydrophilic nature of the starch granule (Vamadevan and Bertoft, 2020). Swelling power and solubility can be used to measure the amount of interaction between starch chains in crystalline and amorphous regions. The magnitude of the interaction is related to the ratio of amylose/amylopectin, phosphor content, amylose/ amylopectin characteristics such as molecular weight, branching or length, and branch conformation (Chan *et al*., 2012). The swelling power and solubility of native and modified starches can be seen in Figure 3.

The swelling power (SP) of modified starch was greater than native starch. The results showed that modified starch with ozone had higher SP than ultrasonication. However, the SP of modified starch with ultrasonication at an amplitude of 80% for 30 mins was almost equal to modified starch with ozone at a slurry concentration of 1:10 with a dissolved ozone concentration of 1.4 ppm. The difference in SP was related to the speed of depolymerization of starch polymers to form carbonyl and carboxyl groups during the oxidation process. The increase in SP can be possibly due to the presence of carboxyl groups and a repulsive force between negative charges which can improve the hydration ability and easily bind hydrogen in water molecules (Pranoto *et al*., 2021; Sumardiono *et al*., 2021). The ultrasonication process was able to depolymerize starch but lacked in producing carboxyl groups that were hydrophilic and positively correlated with the SP. Therefore, modified starch with ozone showed a higher SP than ultrasonication.

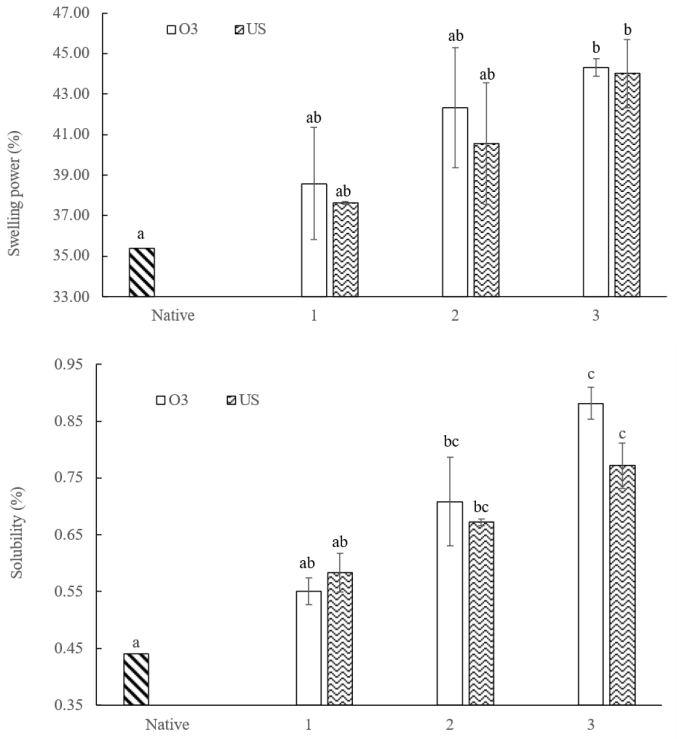

Figure 3. Swelling power and solubility of native and modified starch. Bars with different notations of the same parameter are statistically significantly different at 5% probability level. X-axis 1: starch slurry ratio 1:6 - 0.8 ppm ozone (O_3) and ultrasonication (US) 60% amplitude for 40 mins, x-axis 2: starch slurry ratio 1:8 - 2 ppm ozone and ultrasonication 80% amplitude for 10 mins, x-axis 3: starch slurry ratio 1:10 - 1.4 ppm ozone and ultrasonication 80% amplitude for 30 mins.

The modified starch with ozone and ultrasonication had a significant effect on the solubility of the starch. Solubility represents the amount of solubilized starch molecules at a certain temperature (Wang, 2003). The solubility of modified starch with ultrasonication increased with the increase of amplitude and time duration. Likewise, the solubility of modified starch with ozone increased with the increase of slurry and dissolved ozone concentration. The increase in solubility could be related to the depolymerization and structural weakening of starch granules due to the oxidation process (Adebowale *et al*., 2002).

3.4 Water holding capacity

Water holding capacity (WHC) is defined as the ability of starch to hold water molecules or the amount of water absorbed per gram of sample. The WHC was measured at room temperature and did not indicate the behavior of the starch when heated (Kolawole *et al*., 2013).

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Some studies argued that WHC is related to the weakening of the starch structure due to the breaking of glycosidic chains and the presence of hydrophilic groups formed during the oxidation process such as carboxyl (Lee *et al*., 2005; Uzomah and Ibe, 2011; Pranoto *et al*., 2021). However, in this study, WHC was probably related to a different proportion of crystalline and amorphous regions within the granule. Thus, starch granules with a smaller proportion of weakly bonded amorphous material would presumably imbibe less water (Kolawole *et al*., 2013). Modified starch with ultrasonication has an amorphous tendency which showed a lower relative crystallinity (RC) than modified starch with ozone. The WHC of native and modified starches with ozone and ultrasonication are shown in Figure 4.

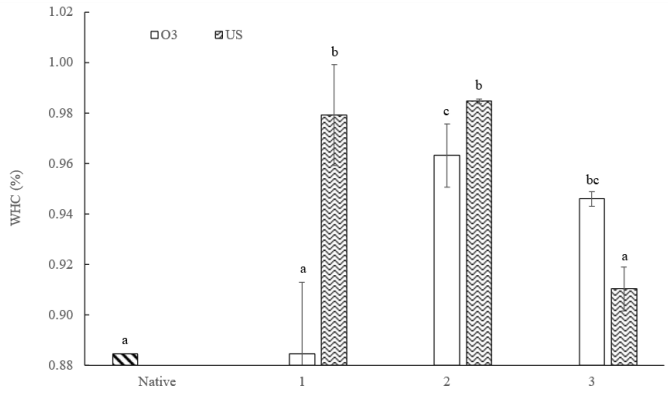

Figure 4. Water holding capacity (WHC) of native and modified starch. Bars with different notations of the same parameter are statistically significantly different at 5% probability level. X-axis 1: starch slurry ratio 1:6 - 0.8 ppm ozone (O_3) and ultrasonication (US) 60% amplitude for 40 mins, x-axis 2: starch slurry ratio 1:8 - 2 ppm ozone and ultrasonication 80% amplitude for 10 mins, x-axis 3: starch slurry ratio 1:10 - 1.4 ppm ozone and ultrasonication 80% amplitude for 30 mins.

3.5 Brightness

Brightness can be represented as the high value of L* in the color measurement. According to Clerici *et al*. (2011), Felisberto *et al*. (2017), and Schmiele *et al*. (2015), commercial starch granules are white and opaque. Starch extraction in laboratory conditions (without oxidizing reagents or enzyme inhibitors) appeared yellow to green due to the presence of pigment or browning enzymatic, like starch from a wolf fruit, chestnut, and bamboo culms. The brightness of native and modified starches by ozone and ultrasonication is shown in Figure 5.

Oxidation with ozone and ultrasonication significantly increased the L* value up to 90-94%, greater than native starch. Brightness increased with the increase of slurry and ozone concentration, as well as the ultrasonication process (the greater amplitude enhanced brightness value). Ozone decomposed into OH radicals. The ultrasonication also could produce OH radicals as the result of the breakage of water molecules. According to Prabaharan and Rao (2001), OH radicals have an important role as bleaching agents in cotton. A previous study also suggested that cheddar cheese whey solution treated with ozone was brighter than the untreated sample (Kang *et al*., 2012). The brightness in starch could be related to the oxidation of starch carotenoid. The OH radicals impair the double bond of carotenoid, resulting in a brighter color. The brightness of ultrasonicated starch decreases at the amplitude of 80% and time duration of 30 mins. It can be probably due to the abundant OH radicals that bind each other, thus the oxidation ability is not effective in oxidizing carotene (El -Sheikh *et al*., 2010).

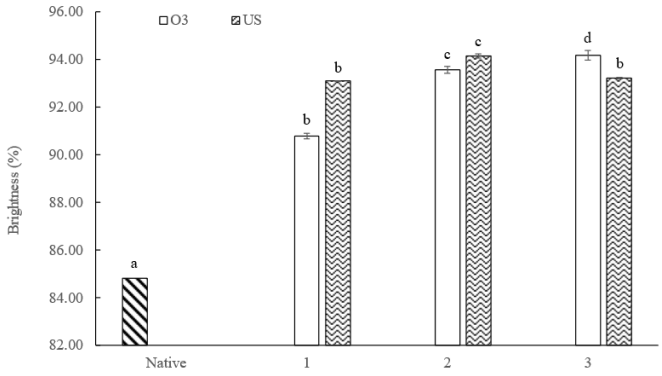

Figure 5. Brightness of native and modified starch. Bars with different notations of the same parameter are statistically significantly different at 5% probability level. X-axis 1: starch slurry ratio 1:6 - 0.8 ppm ozone (O_3) and ultrasonication (US) 60% amplitude for 40 mins, x-axis 2: starch slurry ratio 1:8 - 2 ppm ozone and ultrasonication 80% amplitude for 10 mins, x-axis 3: starch slurry ratio 1:10 - 1.4 ppm ozone and ultrasonication 80% amplitude for 30 mins.

3.6 Pasting properties

Oxidation is always accompanied by hydrolysis of glycosidic bonds within the starch granule. It can lead to depolymerization resulting in lower molecular weight. Therefore, oxidized starch provides low viscosity (Wang, 2003; Takizawa *et al*., 2004; Mottin Demiate *et al*., 2005). One of the parameters of starch modification is the decrease in viscosity. The results showed that the viscosity of the paste decreased for both oxidation with ozone and ultrasonication. Ozone treatment of starch with the slurry concentration of 1:10 and ozone concentration of 1.4 ppm produced the lowest paste viscosity (6083 cP) which is lower than ultrasonication at the amplitude of 80% with 10 mins time duration (6159 cP). The paste viscosity of native and modified starch is shown in Figure 6.

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The concentration of slurry in oxidation with ozone significantly affected paste viscosity. Contact of starch particles with dissolved ozone is more effective at low starch slurry concentrations. The amplitude and time duration in ultrasonication also provide the same result. However, ultrasonication was better in reducing the paste viscosity which can be related to the depolymerization of starch mainly in amylopectin branches resulting in a straight structure with lower molecular weight and an increase in amorphous region. The application of ultrasonication to the liquid system causes acoustic cavitation, which is a phenomenon of the formation of bubbles, growth, and molecular breaking. Cavitation is the breakdown of microbubbles and spreads when sound waves pass through a solution. The energy produced from the cavitation process can break covalent bonds in polymeric materials (Czechowska-Biskup *et al*., 2005; Zhang *et al*., 2013). It was reported that the viscosity of the starch solution and molecular weight of starch decreased after ultrasonic treatment (Iida *et al*., 2008). Pasting involves the swelling of starch granules during heating and the breakage of starch granules leads to the decrease of viscosity in further process. The decrease of viscosity takes place rapidly when starch granules are degraded and can bind the water easily (Kumar and Khatkar, 2017).

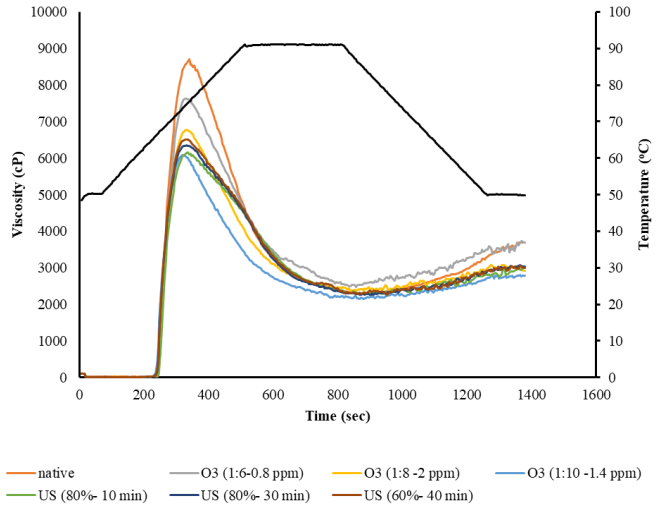

Figure 6. Paste viscosity of native, modified starch with ozone, and modified starch with ultrasonication.

3.7 Fourier-transformed infrared spectroscopy

The majority of chemical groups from native starch are divided into the main groups of C-H, C=C, O-H, and C-O. The peak at 1077 cm^{-1} could be attributed to C-O stretching. The peaks at 2932; 927 and 859 cm^{-1} were assigned as C-H stretching and C-H bending vibrations respectively. The C=C was shown at 1643 cm^{-1}, and O-H bond stretching was at 3300-2500 cm^{-1}. The FTIR spectra of ozone and ultrasound cassava starch are shown in Figure 7a and Figure 7b.

FTIR spectra showed an increase in the intensity of the presence of a new group, the carbonyl group as an indicator of starch oxidation. The absence of a new peak indicated this improper functioning at about 1700 cm^{-1}, which is generally assigned as a carbonyl group (C=O) (Halal *et al*., 2015). According to Petit and Puskar (2018), carbonyl groups generally appear at 1680 to 1780 cm^{-1}. The appearance of carbonyl groups in modified starch with ozone and ultrasonication was different from native starch which is shown by the sharp peak at the wavelength of 1680 to 1780 cm^{-1}. A previous study conducted by Sumardiono *et al*. (2021) also showed the appearance of C=O groups in the oxidized starch. Cassava starch was oxidized with ozone and ultrasonication through the abundant OH radical formed from water hydrolysis. However, oxidation using ozone took place more intensely than ultrasonication. This could be related to the formation of carbonyl groups indicated by the presence of a new peak as seen on the FTIR of the sample.

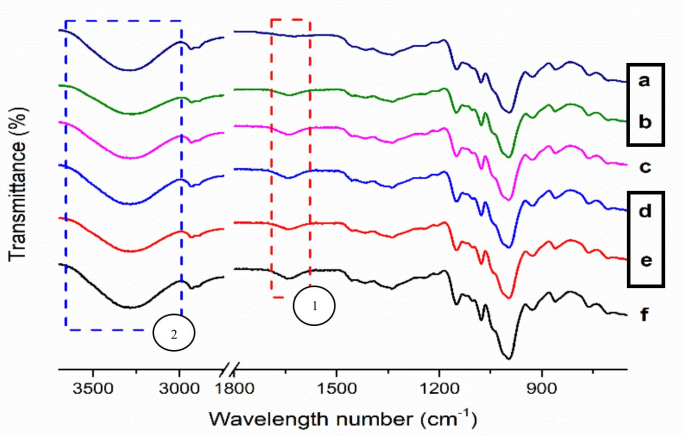

Figure 7a. FTIR spectra of native and oxidation with ozone starch: (a) native, (b) 1:6 -0.8 ppm, (c) 1:8 -0.8 ppm, (d) 1:8-2 ppm, (e) 1:10-1.4 ppm, (1) carbonyl groups (C=O) at wavelength 1680-1780 cm^{-1}, (2) hydroxyl groups (O-H) at wavelength 3300-2500 cm^{-1}.

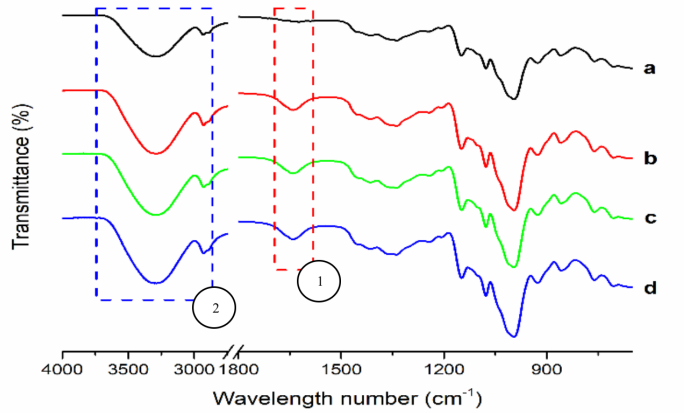

Figure 7b. FTIR spectra of native and ultrasonication starch: (a) native, (b) 60% -40 min, (c) 80%-10 mins (d) 80%-30 mins, (1) carbonyl groups (C=O) at wavelength 1680-1780 cm^{-1}, (2) hydroxyl groups (O-H) at wavelength 3300-2500 cm^{-1}.

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3.8 X-Ray diffractions

X-RD can determine the crystallinity of starch granules. Typically, the degree of crystallinity of native starch granules ranges from about 15% to 45% depending on the source of the starch and is directly related to the amylopectin content and chain length, as well as inversely proportional to the amylose content (Cheetham and Tao, 1998; Hoover, 2001). Starch granules are analyzed in the native form, and the crystalline pattern may change or even disappear in cases of modification reactions. The X-ray diffraction patterns and RC values of the native and modified cassava starches are presented in Figure 8.

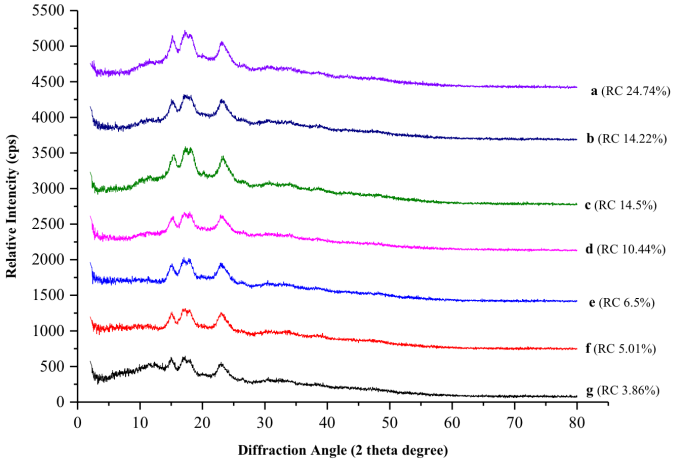

Figure 8. The X-Ray diffraction of native and modified starch: (a) native, (b) ozone 1:6-0.8 ppm, (c) ozone 1:8-2 ppm, (d) ozone 1:10-1.4 ppm, (e) ultrasonication 80% -10 mins, (f) ultrasonication 80%-30 mins, (g) ultrasonication 60%-40 mins.

Modification of starch using dissolved ozone and ultrasonication produced starches with a lower degree of crystallinity than native starches (24.74%). Oxidation with ozone reduces the degree of crystallinity of native starch up to 10.44% at a combination of 1:10 slurry concentration with 1.4 ppm dissolved ozone. Meanwhile, ultrasonication decreased starch crystallinity up to 3.68% at a combination of 60% amplitude with 40 mins time reaction which is lower than oxidation with ozone. This can be explained by the production of cavity and microbubbles in solution by the sonication process. High energy is released during the spreading of microbubbles, and it is converted to high temperatures and pressures. This process causes the degradation of the polymer as well as the acceleration of catalytic reactions (Majid *et al*., 2015). As a result of this treatment, the polymer chains near the collapsing microbubbles are caught in a high gradient shear field, which leads to the breakage of macromolecular C-C bonds, and the formation of long-chain radicals (Monroy *et al*., 2018). Ultrasonication can degrade starch polymers through the phenomenon of cavitation and make them more amorphous with a relatively low degree of crystallinity. The X-ray diffraction analysis results of modified starch with ultrasonication are in good agreement with the amylose content which had higher amylose content than modified starch with ozone with a tendency to form more amorphous starch.

3.9 Scanning electron microscopy

Generally, tuber starch, including cassava has a round shape. Similar to other studies, the granular surface of native starch was smooth and relatively free from imperfections as normally observed in native starch granules (Klein *et al*., 2014; Vanier *et al*., 2017). In contrast, the granules morphology of modified starch with ozone showed rough surfaces and porous shapes (Figure 9). Oxidation with ozone caused the production of irregular starch granules. The ozone concentration of 0.8 ppm with slurry concentration of 1:6 demonstrated a morphological change on starch granule surfaces. Irregular shapes were increasingly observed as increasing in dissolved ozone concentrations. This finding is consistent with the results of functional properties, such as swelling degree and solubility, which were greater in more porous starch granules.

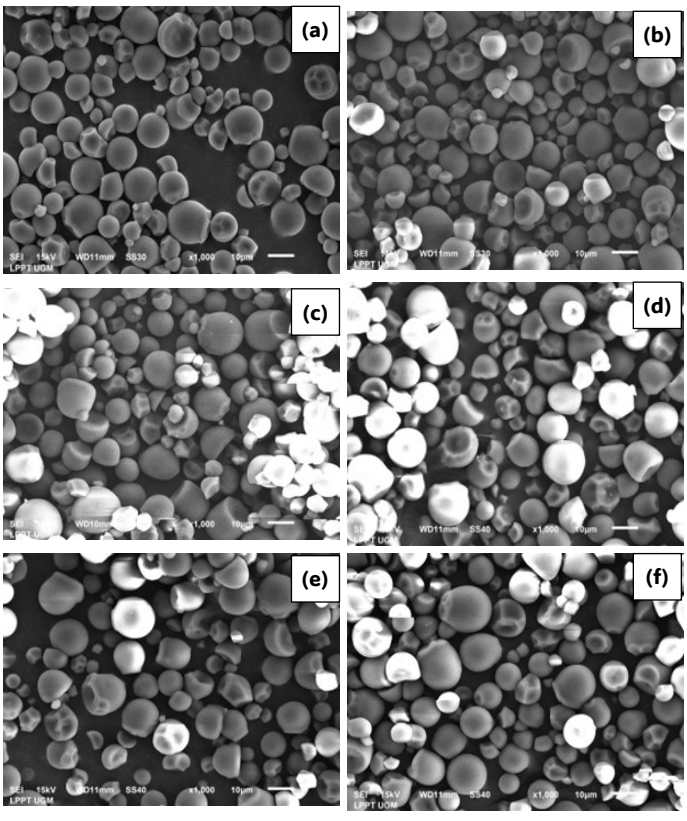

Figure 9. SEM of modified starch with ultrasonication and modified starch with ozone (magnification 1000×): (a) US (60% ampitude-40 mins), (b) US (80% amplitude-10 mins), (c) US (80% amplitude-30 mins), (d) O3 (1:6-0.8 ppm, (e) O3 (1:8-2 ppm, (f) O3 (1:10-1.4 ppm).

The ultrasonication process, according to many reports, causes physical degradation of the granules by forming gaps and pores that appear on the surface.

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Ultrasonication with an amplitude of 60% with 40 mins time duration changed the surface of the starch granules. The SEM image showed that the amplitude and time reaction generated the change in the surface shape of the starch granules. The surface shapes of the starch granules become irregular at the amplitude of 80% with 10- and 30-mins time duration. The porosity of starch granules affected the chemical reactivity of starch. The existence of pores, channels, and cavities increases the surface, which is potentially available for chemical and enzymatic reactions in the presence of a larger specific surface area and increased susceptibility to amylolysis (Sujka, 2017).

4. Conclusion

Two methods of environmental-friendly starch modification, oxidation with ozone and ultrasonication, affected the functional properties of cassava starch. Ozone has a higher degree of oxidation compared to ultrasonication by which the production of carboxyl groups in modified starch with ozone was higher than ultrasonication. Carboxyl groups are more influential on swelling and solubility parameters. Ultrasonication has a stronger tendency to depolymerize the starch as indicated by the production of higher amylose content which was more amorphous (lower Relative Crystallinity value) compared to modified starch with ozone. Overall chemical methods using dissolved ozone and physical methods using ultrasonication can be applied in modifying starch in accordance with expected goals.

Conflict of interest

The authors declare no conflict of interest.

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